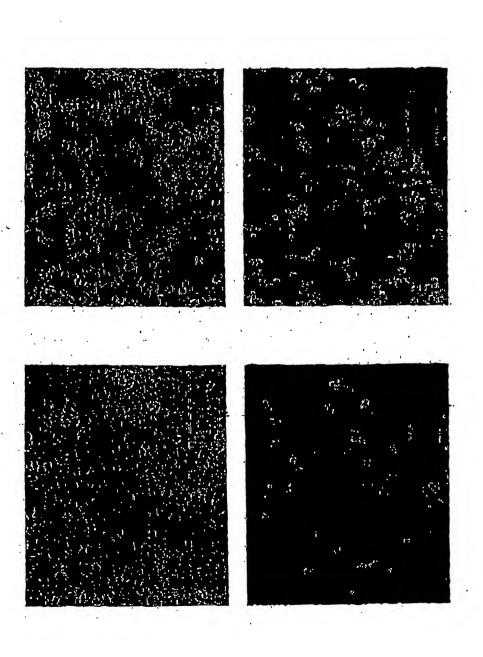
solation of Non-Cytopathic (NCP) VSV Mutants

Select for non-cytopathic variants by screening for adherent GFP (+) cells Obtain plaque isolates, generate stocks and characterize virus After 24 hrs wash monolayer to remove cells exhibiting CPE growth properties, and cell rounding phenotypes Infect cells with rVSV-M33,51A-GFP Collect supernatant and titer (rounded and floating) BHK-21 cells HIK-21 cells Identify cultures that are GFP (+), Maintain culture in incubator Harvest supernatant & Infect fresh cells but that lack CPE

Cell Rounding Phenotypes of M Protein Mutants of VSV



Isolation and Sequencing of NCP-M Protein Mutant cDNAs

Purify NCP virus from culture supernatants of plaque purified NCP isolates

Isolate genomic RNA and perform RT-PCR using M gene specific primers

Subclone cDNAs into plasmid pBS-SIC+

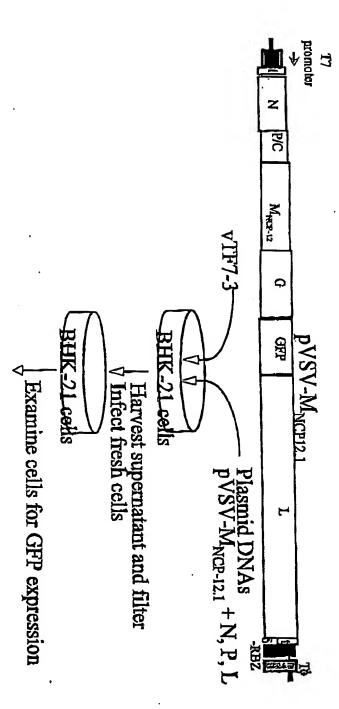
Sequence individual clones and identify mutations present in M_{NCP} cDNAs

NCP mutations are M33A; M51A; T133A; & S226G

Designated M_{NCP12.1}

Subclone M_{NCP12.1} into pVSV-FL(+)-2

Recovery of rVSV-M_{NCP12.1}



Collect supernatant and titer

Obtain plaque isolates, generate stocks and characterize growth properties, and cell roun

rVSV/M_{NCP12.1} is Defective in Cell Rounding

rVSV NCP-12.1 Phase Contrast Anti-VSV N protein MAb Examine @ 12 hrs p.i. Infect BHK-21 cells

Figure 5

Transient Expression of M_{NCP-12.1}

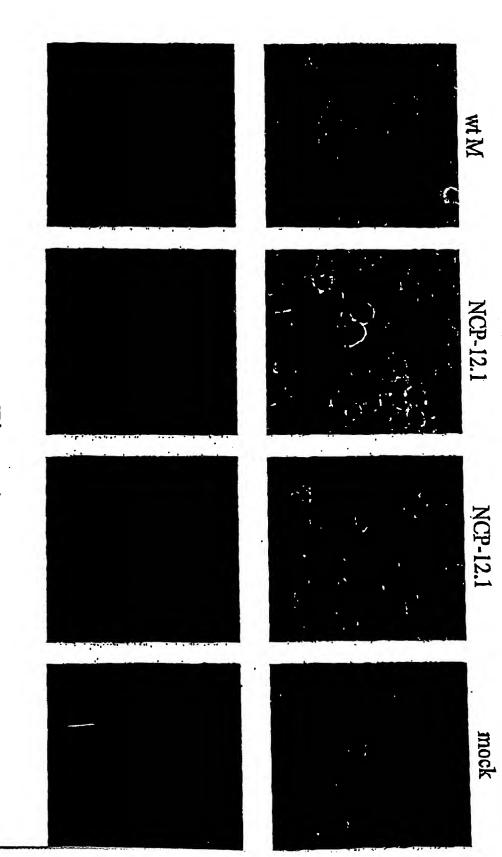


Figure 6

rVSV/M_{NCP-12.1} Infection of Different Cell Types

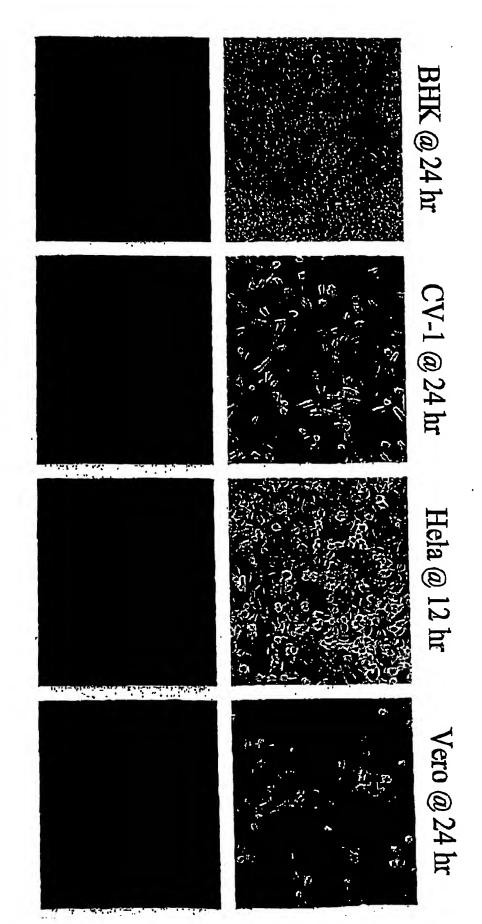
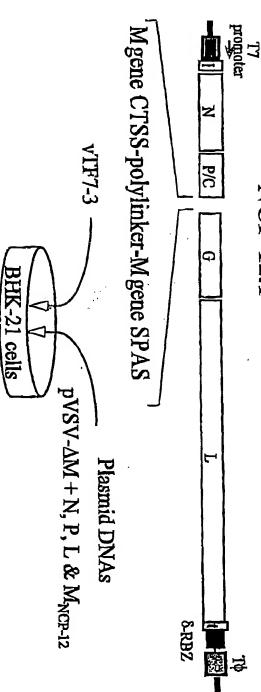
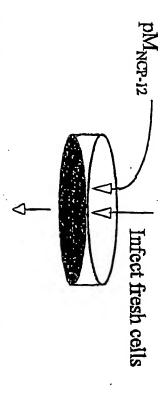


Figure 7

Use of M_{NCP-12.1} to Recover AM-VSV

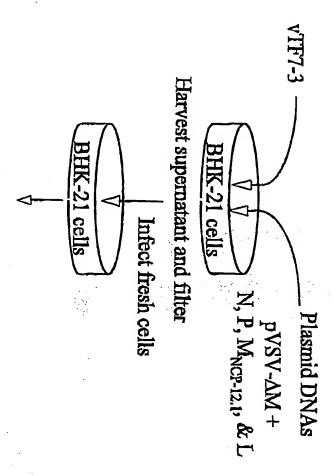


Harvest supernatant and filter

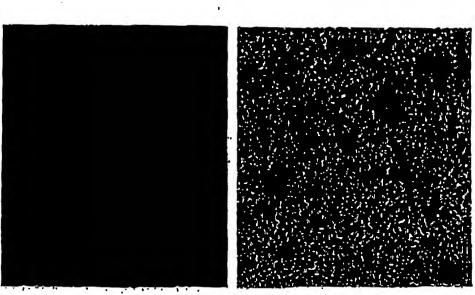


Collect supernatant and examine for N(+) cells

Recovery of rVSV-AM



Examine for N protein expression



Sample #176, 3 Days Post-infection

Infection of islet Prep#176 at day 3 post infection

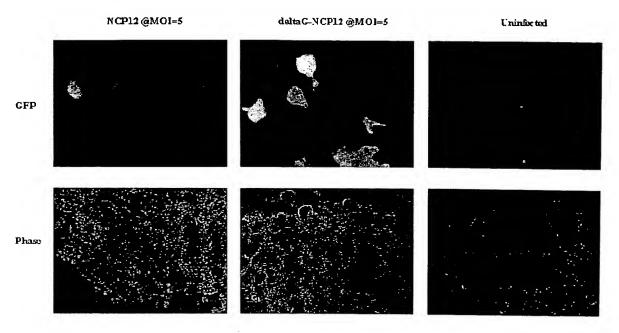


Figure 10

Sample #163, 3 Days Post-infection

Infection of islet Prep#163 at day 3 post infection

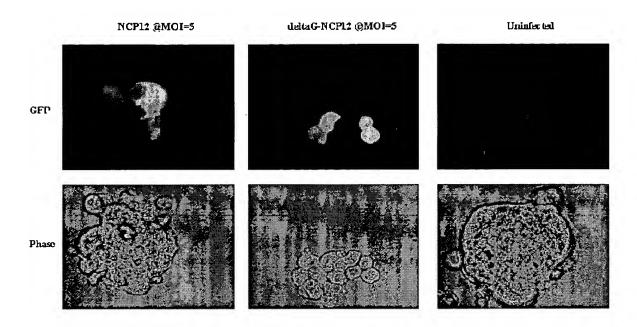


Figure 11

Sample #176, 3 Days Post-infection

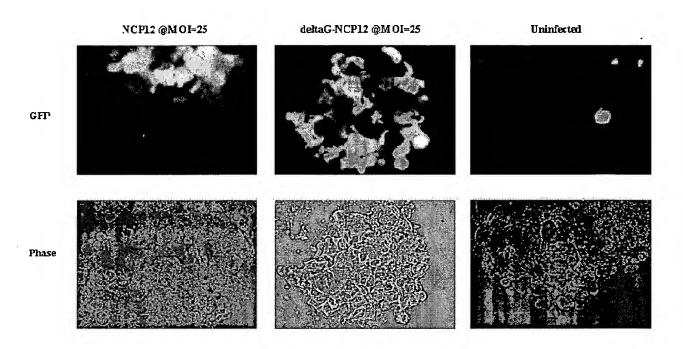


Figure 12

Sample #163, 3 Days Post-infection

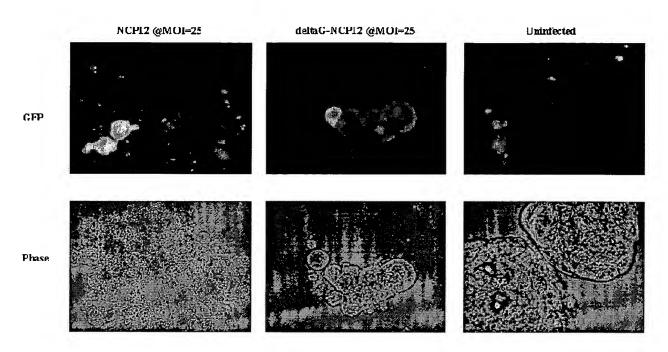


Figure 13

Sample #176, 8 Days Post-infection

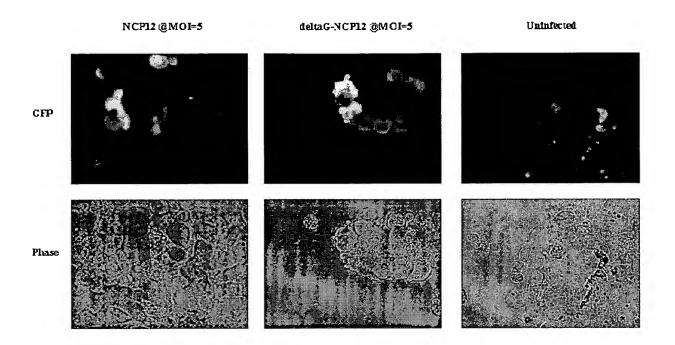


Figure 14

Sample #176, 8 Days Post-infection

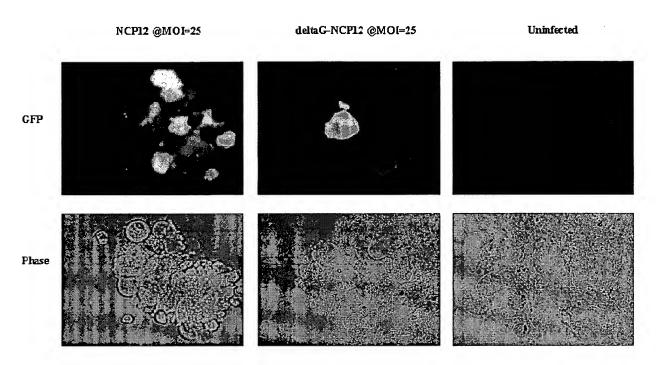


Figure 15

Sample #163, 8 Days Post-infection

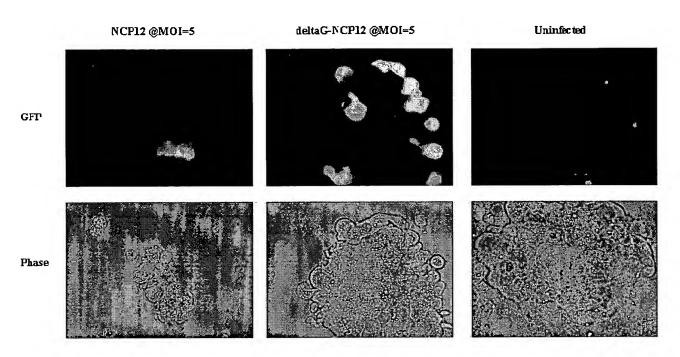


Figure 16

Sample #163, 8 Days Post-infection

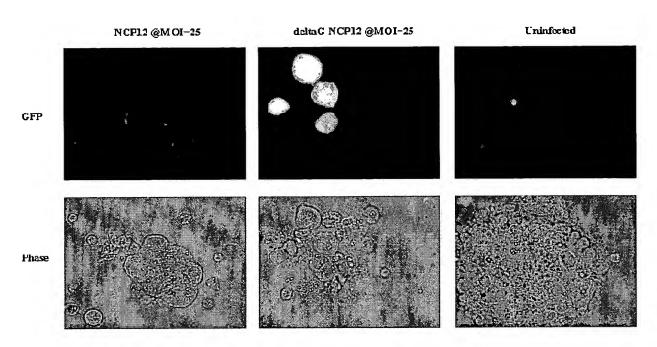


Figure 17

Sample #176, 3 Days Post-infection

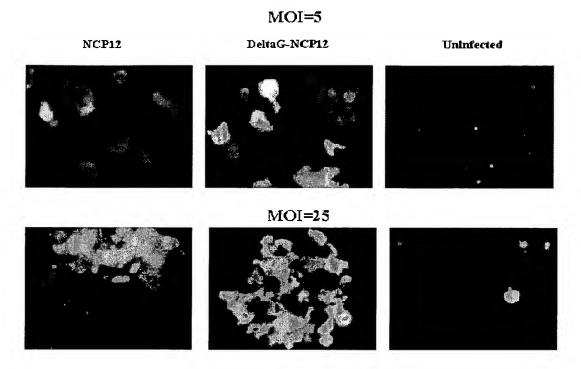


Figure 18

Sample #176, 8 Days Post-infection

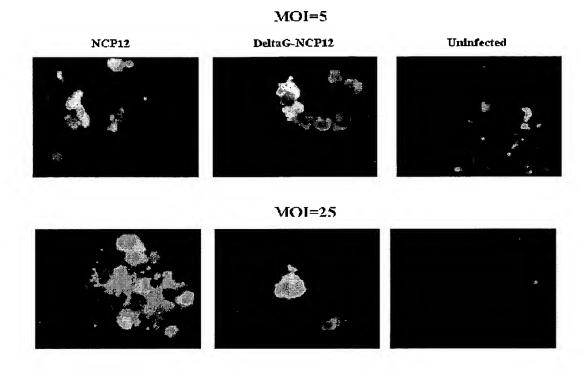


Figure 19

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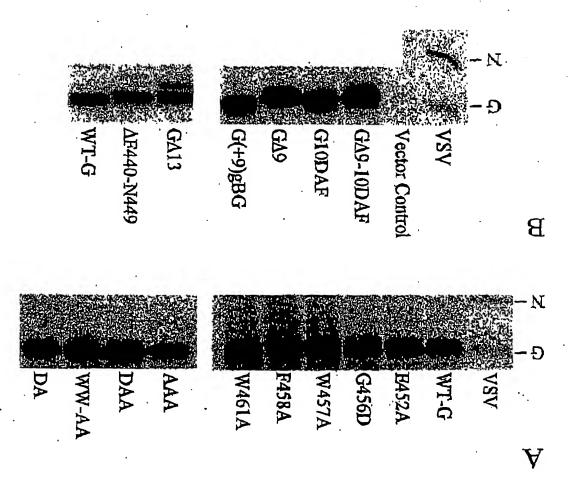
Figure 20

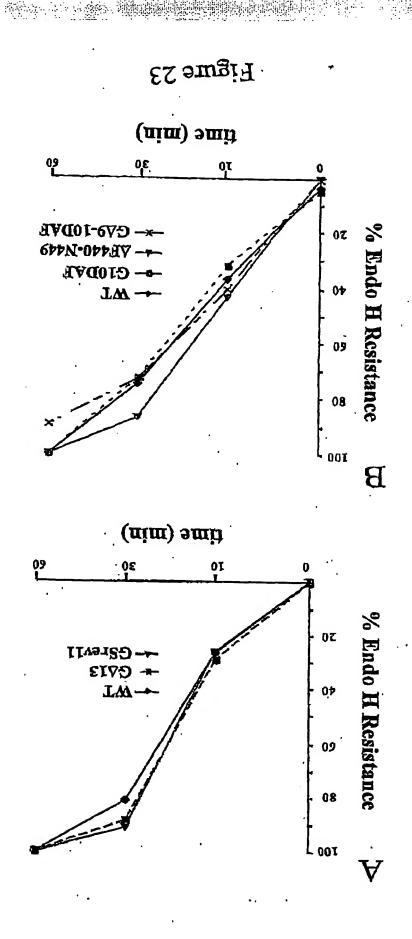
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TM TAIL Grevil	YSÖLPDDESLFFGDTGLSKNPMSSFWGEVLEIK	EEHPHIODA
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W457A(W1A)	¥	
G426D(G-D)	σ	
E4224(E-Y)	₹	

Figure 22





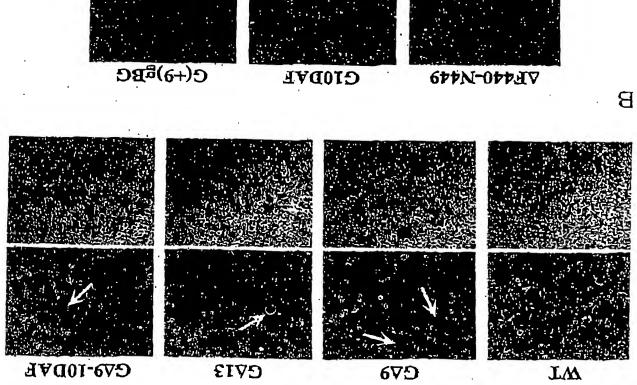
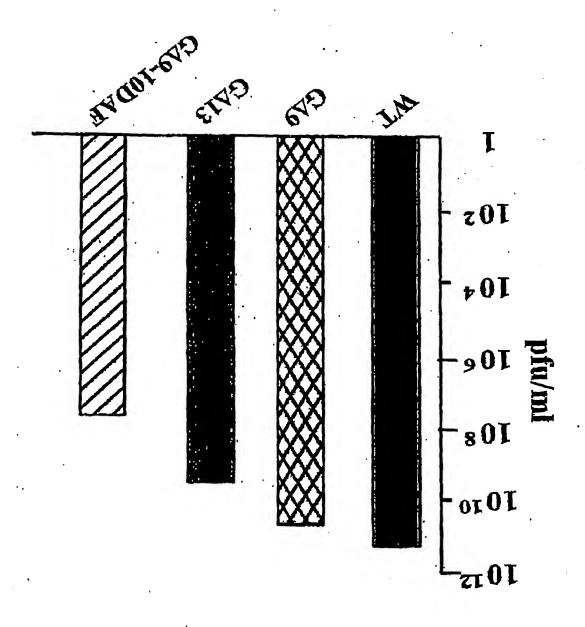
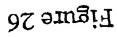
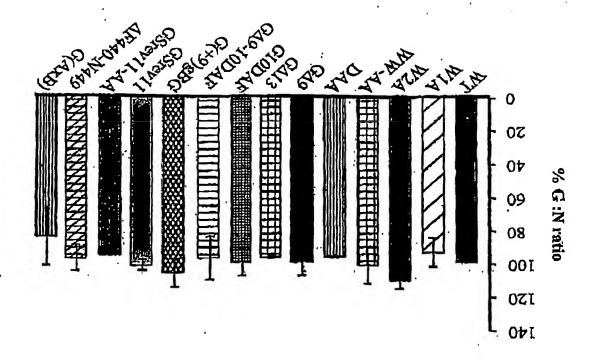
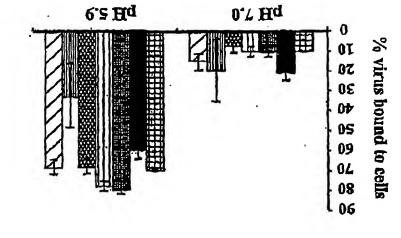


Figure 25









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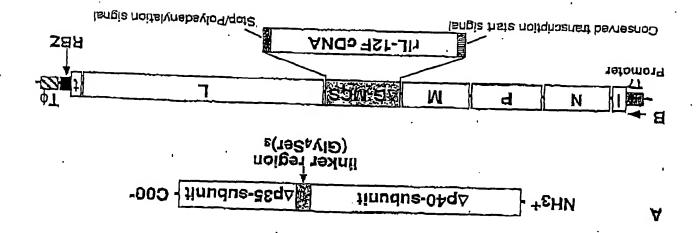
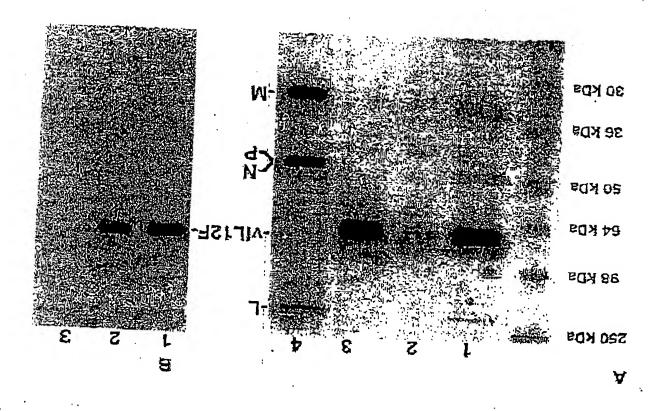


Figure 28

Figure 29



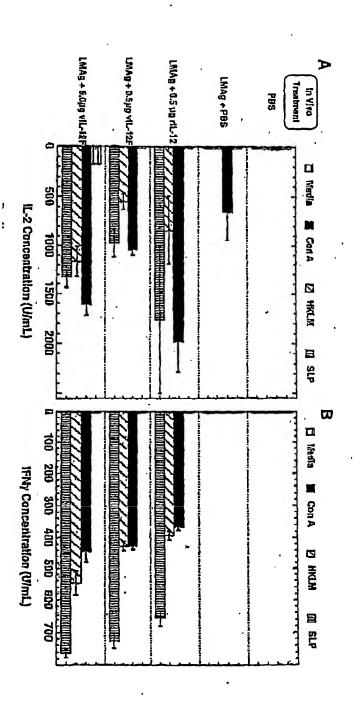


Figure 30

Figure 31

| Liver Colony Counts | Georgadio Log | Mean Frozension | PRS | Liver Colony Counts | Mean Frozension | Debt | De

LNA9 + 5.0 kg vil-12F 5.0 µg vil_12F+ PBS LMAg + 5.0µg v/L-12F 5.0 µg vil-12F + PBS. LIMAg + PBS LMAg+PBS Listeds-Inf. Listeria-int 10° 10° 10° Unalesced Lisberia (CFU) 10° 10' to' Uncleased Listeria (CFU) Liver Colony Counts 4 : Mean Prohector : - 4.29 x 107 0.49 Sacometro Log Mean Protection 1.33 x 100 o 7.83x 105 2.2B 9.31 x 107 Q.SE 3,24 x 105 1,61 1.05 x 108 0.42 4,00x 100 1.84 7.88 × 107 0.85 7.88 x 104 2.55

Figure 33

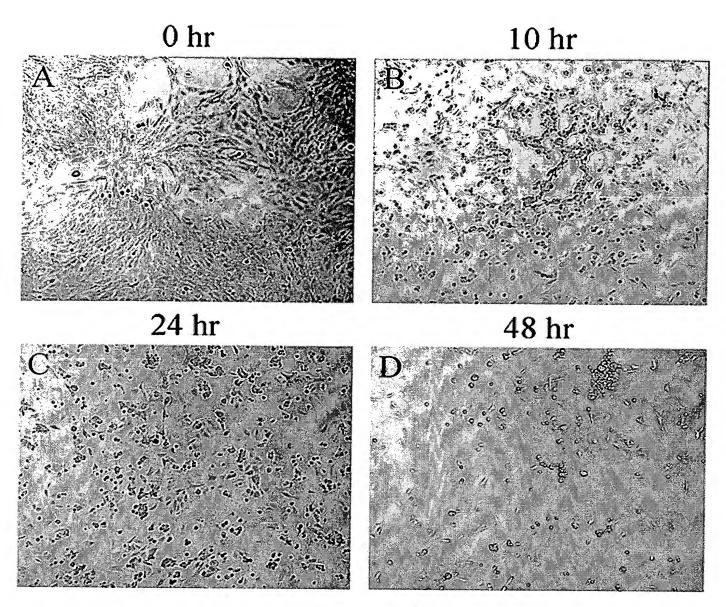


Figure 34

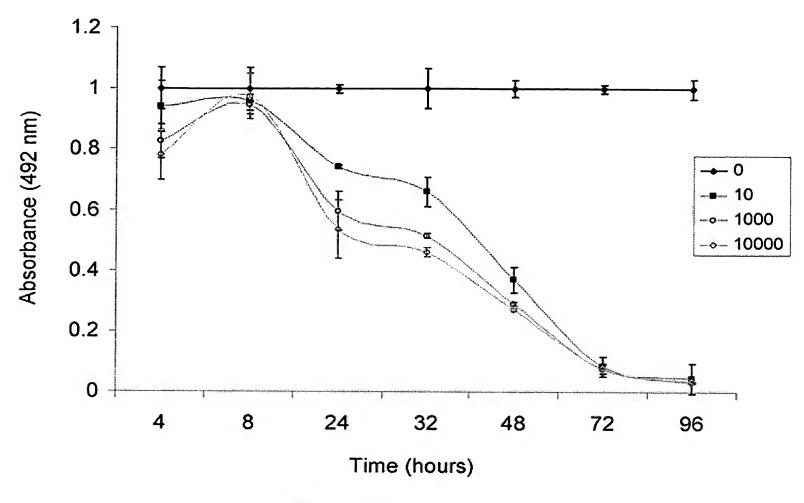


Figure 35

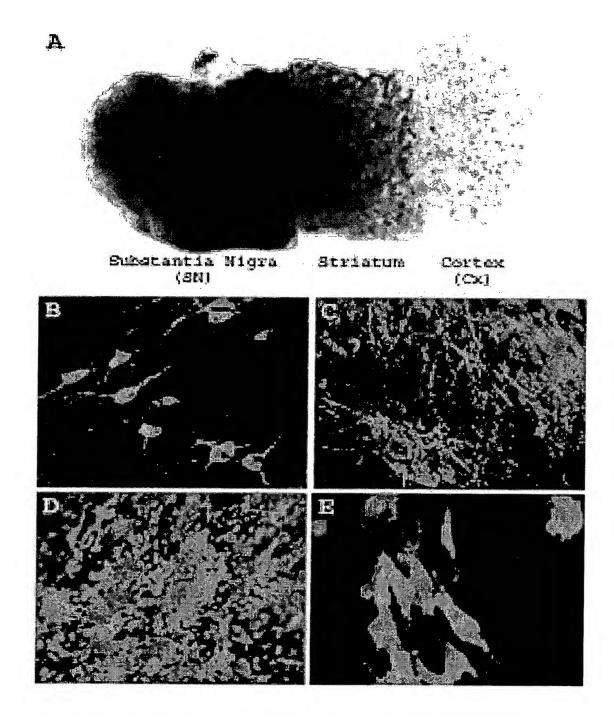


Figure 36

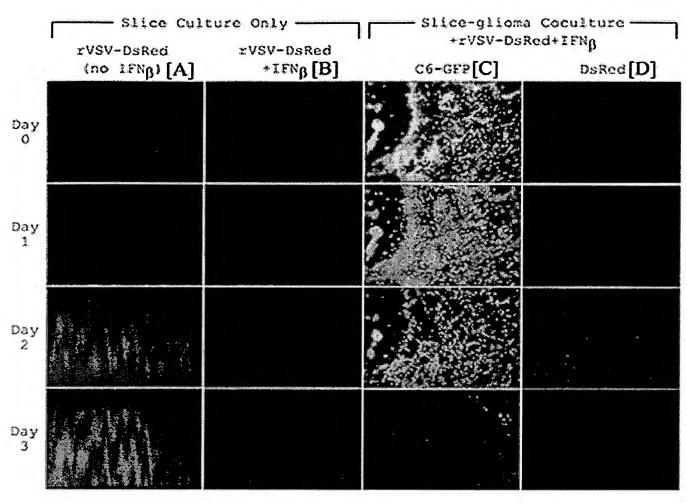


Figure 37

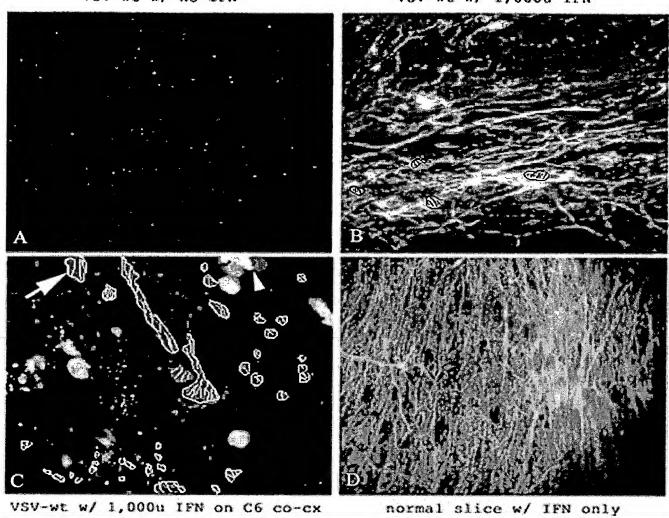


Figure 38

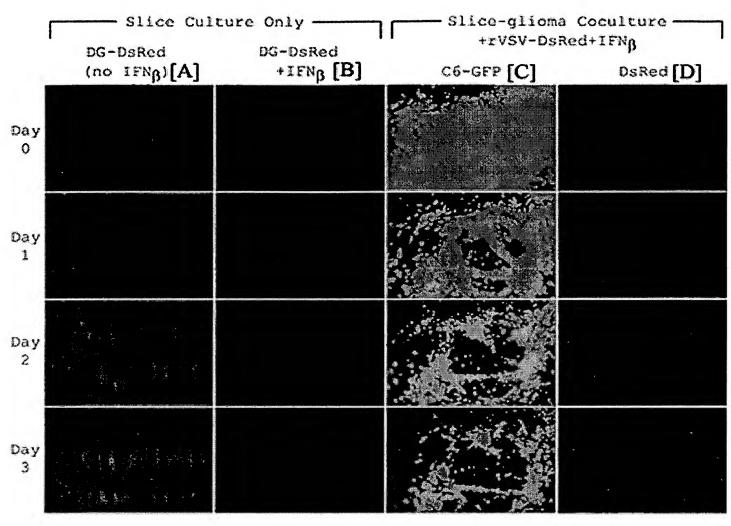


Figure 39

Figure 40

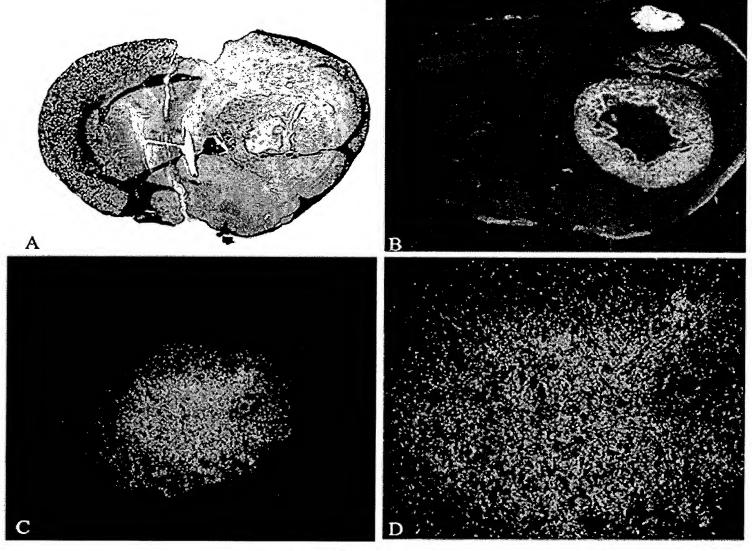


Figure 41

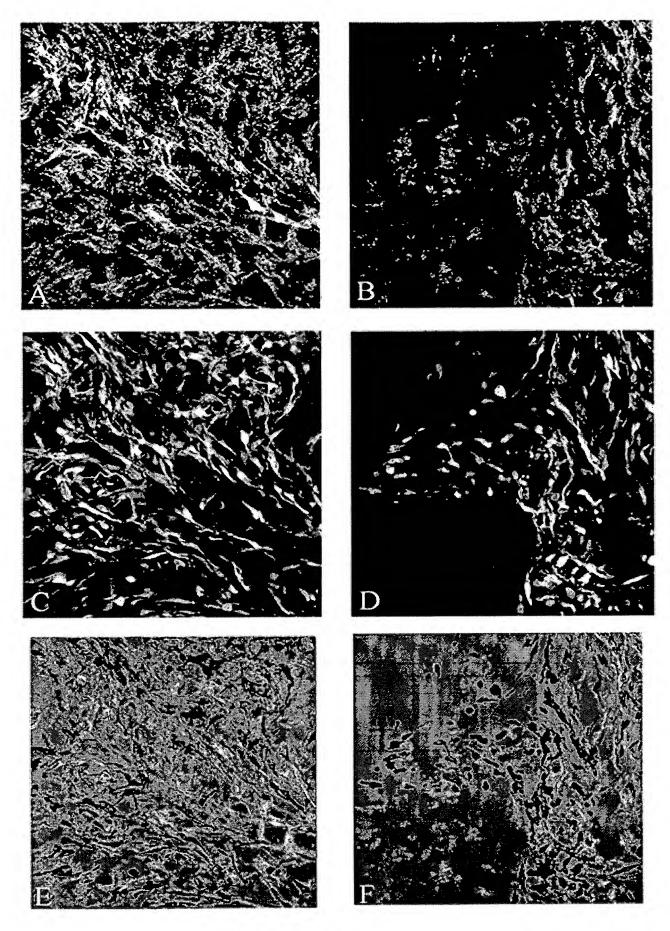


Figure 42